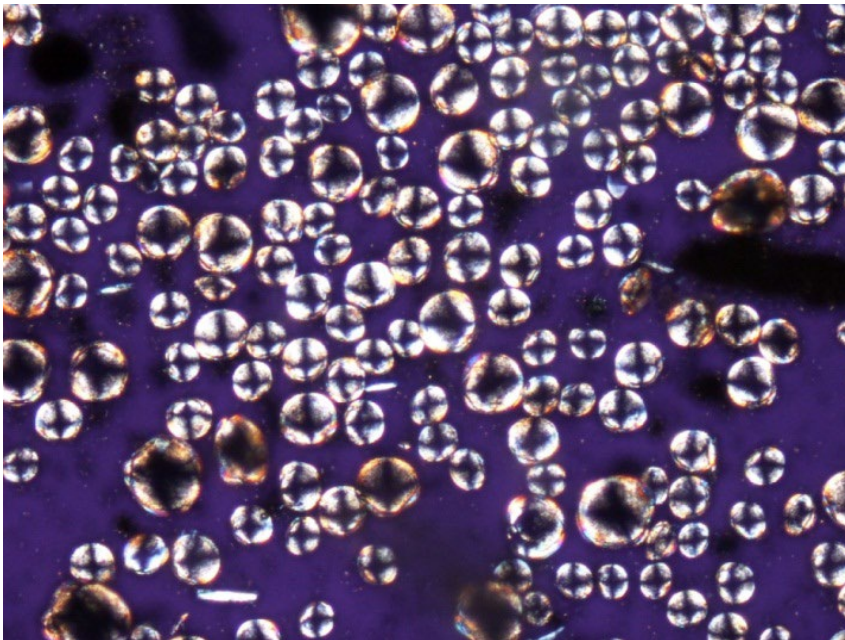




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Alternate Control Strategy for Dreissinids Using Electrical Methods

Science and Technology Program
Research and Development Office
Final Report No. ST-2022-19174-01
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**Bureau of Reclamation
Research and Development Office
Science and Technology Program**

Interim Report ST-2022-19174-01, Technical Report No. 8540-2022-61

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Acronyms and Abbreviations

AC	Alternating current
CAP	Central Arizona Project
CRA	Colorado River Aqueduct
DC	direct current
EPA	Environmental Protection Agency
ERDC	Engineer Research and Development Center
HV	high voltage
MSCP	Multi-Species Conservation Program
O&M	Operations & Maintenance
Reclamation	Bureau of Reclamation
RISE	Reclamation Information Sharing Environment
S&T	Science and Technology
spp.	Subspecies
TSC	Technical Service Center
U.S.	United States

Measurements

°C	degrees Celsius
cm	centimeter
kHz	kilohertz
kV	kilovolts
mg/L	milligrams per liter
mL	milliliters
%	percent
ppm	parts per million
μs	microsecond or 10 ⁻⁶ sec
μs/cm	microsiemens per centimeter
V	volts

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Executive Summary

Zebra and quagga mussels (*Dreissena subsp. (ssp.)*) are major macrofouling species that impact the operations and maintenance of Bureau of Reclamation (Reclamation) water delivery systems. Since they first appeared in the 1980s in the Laurentian Great Lakes, control of these invasive mussel species in North America have focused primarily on the protection of water delivery and industrial systems. Some of the more popular control methods have utilized oxidizing and non-oxidizing chemicals. However, the non-target impact of chemicals on the health of the aquatic ecosystem is a major concern, especially when threatened or endangered species may be present. There is a need for an economical and environmentally safe control strategy for these invasive mussels within Reclamation structures.

The primary objectives of this research project were to perform literature research and determine the feasibility of using electrical methods to mitigate zebra and quagga mussel infestations in Reclamation facilities. This project attempted to carry out a single established electrical testing procedure described in the literature to investigate the effectiveness of electrical control methods under field conditions similar to those found in Reclamation facilities. Multiple methods utilizing electricity have been shown to impact mussel behavior, including mortality and a reduction in the rate of byssogenesis (byssus attachment); however, a single method was chosen primarily on the basis of adaptability to small-diameter pipelines commonly found in Reclamation facilities. The method chosen for testing on this project involved high voltage pulsed electrified fields. Other methods, including direct electrical currents in the media (water), have also been identified in the literature as having the potential to prevent attachments to metallic surfaces, and these may be studied in the future after high voltage pulsed electrical fields have been assessed.

1. Introduction

Zebra and quagga mussels (*Dreissena* spp.) are aquatic, invasive, bivalve species that cause considerable damage to submerged infrastructure involved in the conveyance, treatment, storage, and use of water. Since their initial introduction into the United States (U.S) in the 1980s, they have caused significant problems for utilities and industries in many eastern and central states, particularly in the Great Lakes region and along the Missouri and Mississippi Rivers. Quagga mussels (*Dreissena rostriformis bugensis*) were first detected in Lake Mead in 2007. Since then, the mussels have spread downstream into the Lower Colorado River region and have invaded the Central Arizona Project (CAP) and the Colorado River Aqueduct (CRA), including Lake Mathews, the terminal reservoir on the CRA.

Following initial introduction into an aquatic system, mussels attach to most submerged surfaces, with serious consequences for the drinking water and hydroelectric power industries, industrial cooling facilities, agricultural irrigation, and recreational use of water. Colonies of zebra and quagga mussels clog intake trashracks, pipes, valves, siphons, and irrigation and fire-suppression systems. Consequently, it is critically important to detect infestation in the early stages so that timely and cost-effective response plans and control strategies can be developed.

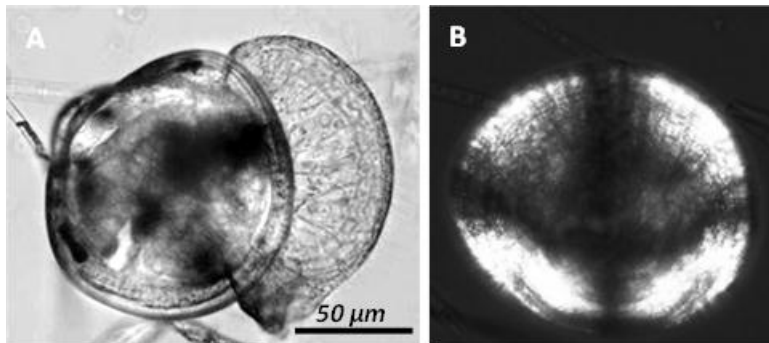


Figure 1. Quagga mussel veligers (larval stage) viewed by microscopy.

A review of the literature has shown that there are a variety of methods for applying electrical energy as a means of sterilizing or pasteurizing products containing microorganisms and zooplankton (Luoma 2017, Smythe 2003, Vega-Mercado 2007). The research described here focused on pulsed electric fields due to its successful commercial use in food pasteurization. There is commercially available equipment that can perform large-scale food pasteurization, indicating that pulsed electric fields is a widely accepted and proven method of inactivating small organisms. The goal of this research then was to determine the feasibility of applying pulsed electric fields to inhibit the passage of live mussel veligers (the larval life stage) within small-diameter raw water pipelines in Reclamation facilities as a means of preventing veliger settlement within these pipelines.

1.1 Project Background

This project proposed to carry out established electrical testing procedures described in the literature to investigate the effectiveness of electrical control methods on mussel veligers under field conditions similar to those found in Reclamation facilities. Alternative methods utilizing electricity have been shown to impact mussel behavior, including mortality and a reduction in the rate of byssogenesis (byssus attachment). Methods include electrified fields, which inhibited passage of live veligers and electrical currents which prevented attachments to metallic surfaces.

A food pasteurization method utilizing homogenous electric fields was found to be effective with 100% lethal effect on bacteria such as *Escherichia coli*, *Staphylococcus aureus*, *Micrococcus lysodeikticus*, *Sarcina lutea*, *Bacillus subtilis*, *Bacillus cereus*, *Bacillus megaterium*, *Clostridium weichii*, and yeasts such as *Saccharomyces cerevisiae* and *Candida utilis* (Vega-Mercado 2007). In general, an increase in the electric field intensity and number of pulses led to an increase of lethal effect or inactivation of microorganisms. For example, an electric field of 19.50 kV/cm at 1020 μ s pulses led to a survival of less than 1% for *Staphylococcus aureus*. The mechanism of cell inactivation was described as a process of cell swelling during application of the electric field, eventually leading to cell lysis (membrane rupture) and inactive cells. Factors that influenced cell or microbial inactivation were treatment temperature, pH, ionic strength, and conductivity of the medium containing the microorganisms. The U.S. Food and Drug Administration found no objection to this approach because it was considered to be an ideal means of aseptic production of safe food without affecting the quality or nutritional value of the food. Other literature indicated the possibility of using electrified fields to inhibit passage and settlement of veligers.

Since pulsed electric fields are successfully used for food pasteurization and pulsed electric field equipment is commercially available, this project investigated using pulsed electrical fields in the past three years. In simple terms, a pulsed electrical field is electrical energy that has been collected at low power levels over an extended period of time and stored in a capacitor. The energy stored in the capacitor can then be discharged almost instantaneously at a very high level of power. The discharge rate can also be controlled as pulses over different intervals of time. A static treatment chamber can be constructed consisting of two electrodes in the configuration of parallel plates held in position, forming a gap between the two plates sufficiently smaller than the surface area of the electrode plates.

The research team decided that creating a pulsed electrical field across a small-diameter raw-water pipeline would also be a more desirable method of control since it doesn't require electrical current across the pipe or raw water itself. Small-diameter raw-water pipelines, such as those normally used in Reclamation powerplants, are often 4-inch diameter or smaller in once-through cooling systems that convey heat from generators and turbines. After a two-year moratorium was lifted on COVID-19 travel restrictions for non-essential activities such as Science and Technology (S&T) research projects, the research team made its first and only attempt to determine the effect of pulsed electrical fields on live quagga mussel veligers in Boulder City, Nevada (NV). Live veligers were collected locally from Lake Mead and subjected to various electrical fields using an apparatus designed and developed by the 86-68400 Electrical & Mechanical Engineering Division. Results from this initial testing did not yield promising results. However, other literature indicated that electrical currents should be applied directly to the raw water media or the metallic surface itself to achieve high mortality or low settlement rates. For

this reason, the research team submitted a new proposal to investigate the direct application of electrical currents to the raw water media to determine the effectiveness of electrical methods in preventing settlement.

1.2 Previous Work Performed

No previous work has been performed by Reclamation using electrical methods to control mussel infestation in Reclamation facilities prior to the work described here from this research project.

Reclamation has performed investigations on the impact of invasive mussels on various metallic features (fish screens, pipes, strainers, stoplogs, gates, trashracks, etc.). These are already well documented within the listing of previous S&T research projects and will not be repeated here. These metallic features may be candidates for electrical controls, either by preventing passages of veligers using an electrified field in a confined space (small pipes, strainers, cooling lines, etc.) or by impressed currents on a metal surface susceptible to biofouling (valves, screens, etc.).

Outside of Reclamation, there are publications on the use of electrical methods to control invasive mussels. Most of the previous studies have focused on the stunning or killing of the planktonic life stages of dreissenid mussels. These studies have shown that a large amount of electricity is not required. 100% mortality or inhibition of byssogenesis have been achieved using currents on a level similar to cathodic protection systems. A few have studied the use of electrified fields on attached adult dreissenid mussels. One recent study completed by the U.S. Army Corps of Engineer Research and Development Center (ERDC) was successful in causing 100% detachment of adult mussels (Claxton 2017). At the same time, no corrosion, pH changes in the water, or other adverse impacts were observed. This was accomplished via design considerations of the electrical control apparatus. This demonstrates that it is possible to utilize electrical control methods without impacting adjacent infrastructure. This is especially true when the feature to be protected is already confined and electrically isolated from all other features of the facility.

In addition to controlling invasive mussels, electrical methods have also been used for sterilization and pasteurization. Applications include ohmic heating, microwave heating, low electric field stimulation, high-voltage arc discharge, and high-intensity pulsed electric field (Vega-Mercado 2007). Of these applications, high-intensity pulsed electric field appeared to be the most adaptable for raw water-cooling pipelines in Reclamation hydropower plants. Since the pulsed electric field technology is more developed and commercially available in the food processing industry, this research project elected to design an apparatus along similar parameters to determine the effectiveness of pulsed electric fields on zebra and quagga veligers. However, these electrical methods of sterilization and pasteurization were typically developed to kill or inactivate microorganisms (bacteria) and enzymes in food products. It is uncertain how effective pulsed electric fields may be on larger multi-cellular organisms with developing shells, which is the study objective of this research.

1.3 Research Partners

No external research partners were involved on this project. Some initial research in this field was performed by ERDC in the past and information of their experimental set up was obtained, but they have yet to continue their research and are not collaborating on this research with Reclamation due to other priorities.

All work described in this research was performed by engineers and scientists from the Technical Service Center (TSC). Electrical engineers from the 86-68450 Hydropower Diagnostics and SCADA Group and the 86-68440 Power Systems Analysis and Control Group designed and constructed the apparatus for testing electric fields on live quagga veligers, as well as assisted with the field testing. Scientists from the 86-68540 Materials & Corrosion Laboratory Group and the 86-68560 Hydraulic Investigations & Lab Services Group carried out the field testing near Lake Mead, NV.

The Multi-species Conservation Program donated laboratory space at their fish laboratories where we conducted field testing of our electrical apparatus. The Lower Colorado Regional Laboratory provided analyses of our water samples for alkalinity and pH measurements. Both laboratories are part of Reclamation's Lower Colorado Basin Region and are located in Boulder City, NV.

1.4 Objectives

A particular application the research team focused on was preventing the passage of live veligers in raw water-cooling pipelines used to cool generators and turbines in Reclamation powerplants. When turbines and generators are used in a hydropower plant to generate electricity, there is also an accompanying increase in the heat generated by the turbines and generators. For this reason, raw reservoir water drawn via cooling lines are often used as cooling water. These cooling lines are relatively small-diameter pipes (e.g., 4-inch diameter) and are susceptible to zebra and quagga mussel settlement and colonization due to their ability to attach to steel surfaces. The planktonic life stage of these mussels are drawn into the cooling pipelines along with the raw reservoir water. These veligers may settle inside the pipe, grow, and create layers of repeated settlements which will eventually become a clogging issue inside the pipe and impact the flow of cooling water. The layers of mussels on the wall inside the pipe also impact the thermal transfer efficiency of the cooling system. This combination of reduced flow due to clogging and reduced thermal transfer efficiency may cause a loss of heat dissipation and a dangerous rise in the operating temperatures of the generators and turbines. Since these raw water-cooling pipelines are traditionally a once-through flow system of raw reservoir water, there is a continuing onslaught of planktonic veligers from the reservoir. This results in increased operations and maintenance (O&M) requirements to keep these cooling lines free of invasive mussel biofouling. In some locations where water temperatures are warm enough year-around, these invasive mussels are spawning year-around, which then requires year-round O&M to keep the performance of these cooling pipelines free of biofouling.

Of multiple electrical methods that were found during literature review, the project team decided that application of an electric field may be the most desirable approach to prevent the passage of live mussel veligers in raw water-cooling pipelines in Reclamation powerplants. An electric field can be generated across the inner spacing of a pipeline. To determine the feasibility of using an electric

field to mitigate the settlement of veligers, a bench-scale apparatus was designed and constructed to supply both direct current (DC) and pulsed electric fields. The methods and results from this work are described below.

2. Summary of Methods and Results

2.1 Methods

2.1.1 Electrical Apparatus

To perform field testing of a pulsed electrical field on live quagga mussel veligers, an electrical apparatus was designed and constructed by TSC engineers in the 86-68450 Hydropower Diagnostics and SCADA Group and the 86-68440 Power Systems Analysis and Control Group. As previously described, the apparatus was designed along similar parameters used for food pasteurization utilizing homogeneous electric fields. Instead of the mass production processing found in food pasteurization, a single petri dish containing live mussel veligers is placed in between two plates across which electrified fields are applied to determine their effect on live mussel veligers.

The Standard Operating Procedure (SOP) for the apparatus is provided in Appendix A. A more abbreviated description of the apparatus set up and operations in both pulsed and DC modes are discussed below.

2.1.1.1 Equipment

Two electrical boxes make up the key components of the apparatus: the high voltage (HV) generator and the test chamber. The HV generator contains all the electronics necessary to generate HV continuously or in pulses. The test chamber contains metal plates that the HV is applied across, and samples of petri dishes containing live mussel veligers can be placed between them. Other components include a function generator, a DC power supply, and an oscilloscope.

The HV generator is depicted in Figure 2. The purpose of the HV generator is to generate the HV signal that is applied to the test chamber.

The test chamber is the second electrical box that makes up the heart of the apparatus. It has multiple cables attached directly to it and large grey tabs that can be rotated to lock/unlock the chamber as shown in Figure 3. The test chamber is where petri dishes can be placed and are subjected to high voltage to study the effects of high voltage pulses on live veligers. The test chamber comes with a built-in safety feature that allows for power to be immediately cut if the test chamber is opened during operation, preventing direct exposure to HV within the test chamber if the HV generator is not first turned off.

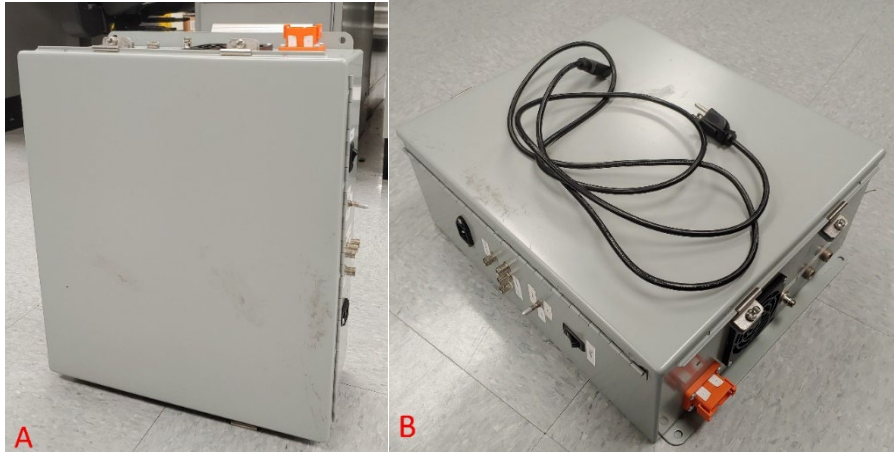


Figure 2. The HV generator. Standing up position (A) and lying down position (B).

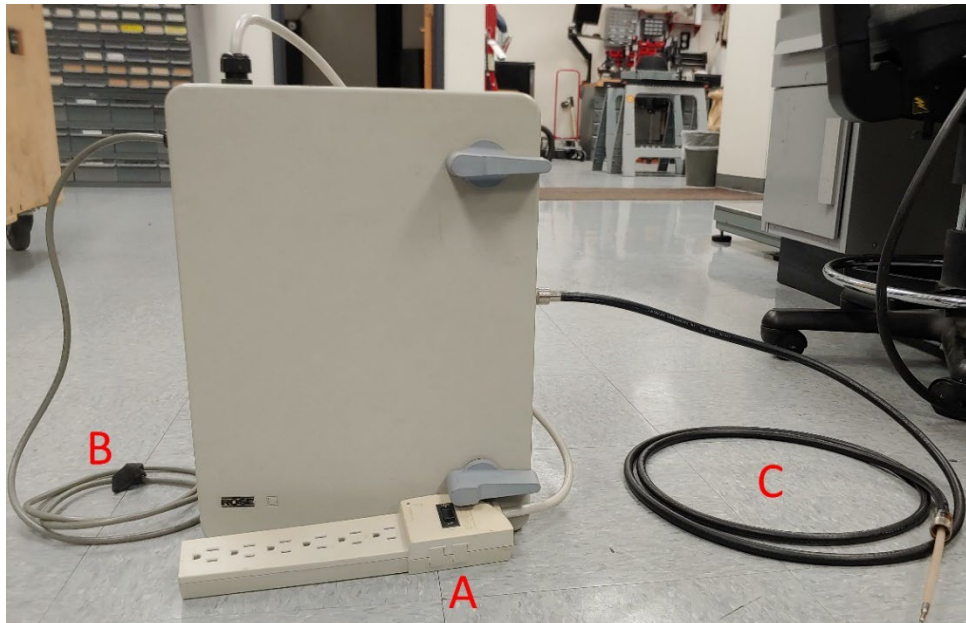


Figure 3. The test chamber from the front side. Note the power strip (A), power cord (B), and high voltage cable (C).

The function generator is used to generate the necessary signal for the HV generator's oscillator circuit to work and is shown in Figure 4. The frequency of the signal generated by the function generator will primarily determine the frequency of pulses in the test chamber. A coaxial cable is used between the function generator output and the PULSE_INPUT terminal of the HV generator.

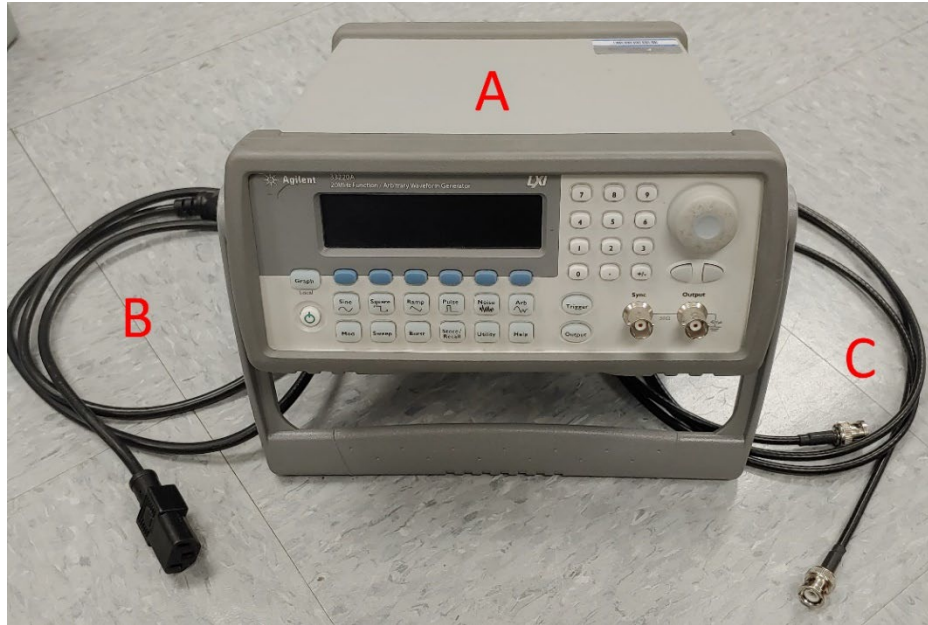


Figure 4. The function generator (A). With its power cable (B) and a coaxial cable (C).

The DC power supply is connected to the HV generator and is the primary power source for the generation of high voltage. Adjusting the voltage output of this source will increase or decrease the magnitude of the voltage generated by the HV generator. It consists of the source itself with two cables connected to it as shown in Figure 5.

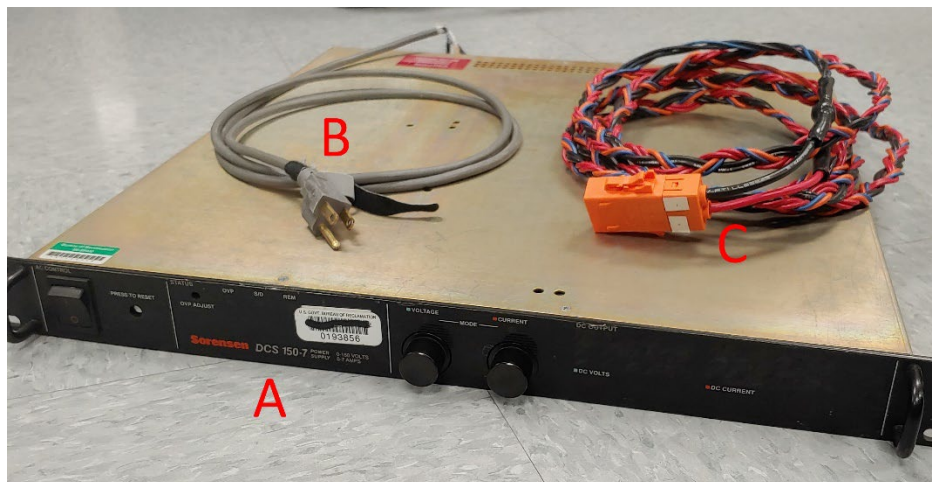


Figure 5. The DC power source (A). With its power cable (B) and the connection to the HV generator (C).

The oscilloscope is used to monitor various signals from the HV generator, including the output, and is shown in Figure 6. The oscilloscope is accompanied by four coaxial cables so that all four channels of the oscilloscope may be used at once as well as an alternating current (AC) test adapter on its power cord. Training on the use of the oscilloscope along with the apparatus is required before a user may understand how the apparatus is working and the magnitude of the output voltage.

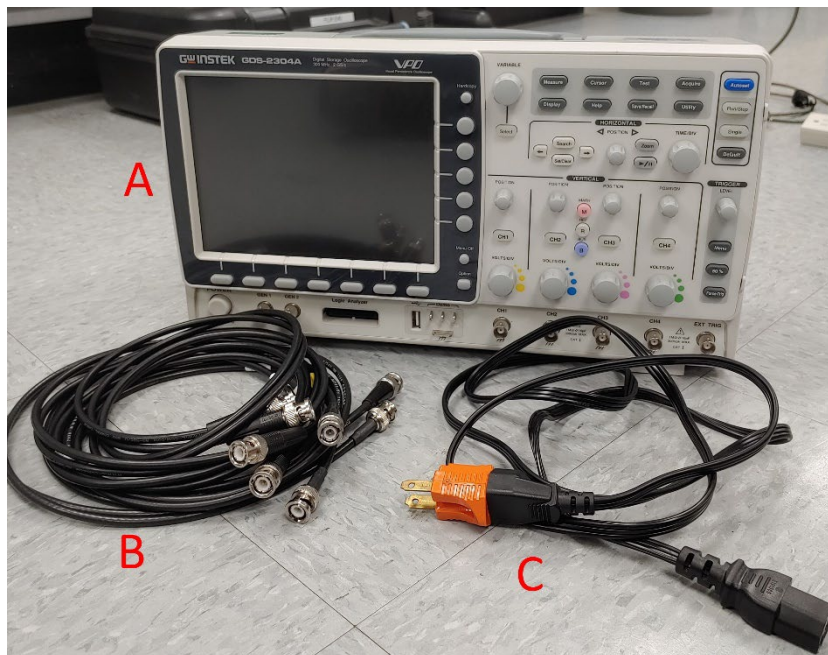


Figure 6. The oscilloscope (A). With coaxial cables (B) and its power cord terminated with an AC test adapter (C).

The entire apparatus sources its power from a single 120-volt (V) wall outlet connection through the test chamber. This allows all necessary components to be plugged into the test chamber surge protector.

2.1.1.2 Operations

After the apparatus is fully set up in accordance with the SOP in Appendix A, the function generator and oscilloscope are used together to determine if the oscillator circuit is working properly, and the desired output is correctly produced. The oscillator circuit may require tuning before proceeding with testing. The oscillator is considered tuned when the astable signal has a frequency in the range of 20 – 25 kilohertz (kHz) and the monostable signal has a pulse length approximately equivalent to one period of the astable signal. The frequency and pulse length of these signals can be measured on an oscilloscope. For more details on working with the oscillator circuit, a separate documentation file *Oscillator Documentation* is available from 86-68450 Hydropower Diagnostics and SCADA Group.

The frequency of the HV pulses is dictated by the frequency from the function generator. Once the function generator output is set to the desired parameters, it may output the signal to the HV generator as a means of controlling the parameters of the HV generator. The HV generator may be operated either in DC or Pulse mode. The oscilloscope is used to determine useful quantities from the HV generator, such as the frequency and voltage output in the test chamber. This provides a means of control and measurement while performing tests on petri dishes containing live veligers. The output voltage will depend on the mode that the HV generator runs in and the input voltage from the DC power supply. Running the HV generator in PULSE mode requires a larger input voltage from the DC power supply to achieve the equivalent peak-to-peak voltage that the DC mode generates. Thus, when running in DC mode, the input voltage from the DC source and the output should be monitored more carefully to ensure the high voltage generated is not too high.

The peak-to-peak voltage of the output can be measured directly on the oscilloscope by using cursors to get information about the magnitude of the output signal.

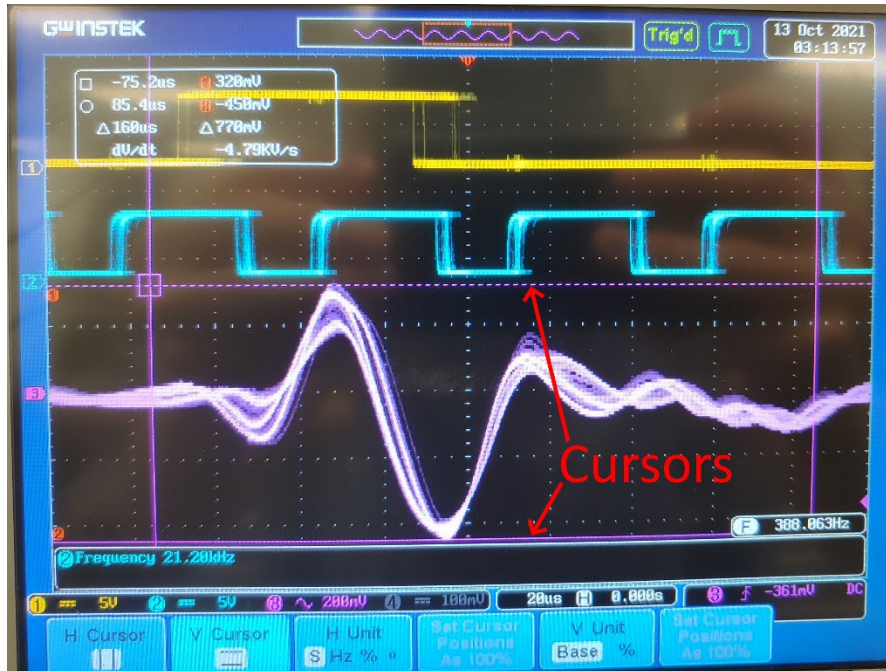


Figure 7. Example of an oscilloscope display. Vertical cursors are used to measure the peak-to-peak voltage of the output signal.

Equation 1 provides the voltage divider relationship between the output voltage and the actual high voltage generated in the test chamber.

$$V_{Chamber} = \frac{V_{output}}{0.000203292} = 12,767 \cdot V_{output} \quad (\text{Equation 1})$$

As an example, Table 1 gives a summary of the voltage generated in both PULSE and DC modes for given DC power supply inputs. The table is used as a reference and not for actual evaluation of the output voltage since it can vary with the oscillator pulse frequency and other unidentified factors. The peak-to-peak voltages given in the four output columns that can be seen on the oscilloscope are the result of a voltage divider. The last column gives the actual voltage generated across the plates in the test chamber. Equation 1 gives the voltage divider relationship between the output and the actual high voltage generated in the test chamber.

Table 1. Parameters related to output voltage. (Astable Frequency = 21.3 kHz, Duty % = 60%)

DC Source Voltage (V)	Pulse Output (Vpp)	Pulse Output Between Plates (kVpp)	DC Output (Vpp)	DC Output Between Plates (kVpp)
1.0	0.0300	0.148	0.076	0.374
5.0	0.109	0.536	0.275	1.353
10.0	0.31	1.525	0.54	2.656
15.0	0.406	1.997	0.8	3.935
20.0	0.52	2.558	1.12	5.509
25.0	0.65	3.197	1.29	6.346
30.0	0.77	3.788	1.44	7.083
35.0	0.941	4.629	Arcing	Arcing
40.0	1.05	5.165	Arcing	Arcing
45.0	1.18	5.804	Arcing	Arcing
50.0	1.3	6.395	Arcing	Arcing
55.0	1.44	7.083	Arcing	Arcing
60.0	1.55	7.625	Arcing	Arcing
65.0	1.67	8.215	Arcing	Arcing
70.0	1.75	8.608	Arcing	Arcing

2.1.2 Veliger Analysis

The veligers that were used in this study were collected from the Las Vegas Boat Harbor at Lake Mead (36°01.927' N, 114°46.100' W) each morning before testing began. Only quagga mussels have been found in Lake Mead. Water quality was assessed at three depths (surface, mid, and bottom) at the location where veligers were collected using a Yellow Springs Instruments (YSI) multi-probe. Additionally, a surface grab sample was collected and analyzed by the Lower Colorado Regional Lab for alkalinity. Veligers were collected with a 64- μ m plankton tow net and were transported in Nalgene bottles to the Reclamation Multi-Species Conservation Program (MSCP) Fish Lab in Boulder City, NV.

The veligers selected for use in tests were confirmed to be alive by observing velum movement under the microscope. Each test of the electrical apparatus consisted of three replicate petri dishes containing 15 veligers each. Each petri dish contained 11 milliliters (mL) of Lake Mead water and five d-stage, five umbonal, and five pedi-veligers. Veligers of each life stage were included to determine if the electrical treatment affected each larval stage differently. For each test, an additional three control petri dishes were prepared that contained the same veliger concentrations. The control veligers were not exposed to the electrical treatment. The control veligers were observed alongside the treated veligers to detect any underlying mortality that was not related to the treatment. A total of 45 veligers were exposed to each of the six electrical treatments, and a total of 45 veligers were observed as controls for each treatment.

For each test, a single petri dish was placed in the electrical apparatus for the appropriate exposure duration. Veligers were analyzed immediately after exposure for mortality or behavioral changes. The same treated veligers and control veligers were also analyzed one hour and 24 hours post

exposure time. Each veliger was examined under the microscope and was determined to be alive when movement of the velum or other organs was observed. In between observations, both treated and control petri-dishes/veligers were placed in a 17 degrees Celsius (°C) water bath to maintain temperature. The temperature was selected based on American Society for Testing and Materials (ASTM) toxicity testing standards that recommend water bath temperature should be within 5°C of the temperature of water from which the test organisms were obtained. The final test (test 6, Table 3) only included one replicate and was conducted on the last day of the study, so there was not enough time to analyze the veligers after 24 hours. Therefore, veligers in this test were only observed immediately and one hour after exposure.

2.2 Results

2.2.1 Water Quality

As described, an in-situ multi-probe water quality instrument from YSI was used to measure common water quality parameters at the same location where live quagga mussel veligers were collected (Las Vegas Boat Harbor, Lake Mead). Water samples were also collected and returned to the Lower Colorado Region Laboratory (Boulder City, NV) to measure alkalinity using Standard Method 2320 (APHA 1992). The YSI results are included in Table 2 and the alkalinity related results are in Table 3.

Table 2. YSI water quality at three depths at Las Vegas Boat Harbor, Lake Mead

Parameters	Surface	Mid-depth	Bottom
Depth (meters)	0.6	9.5	19.4
Temperature (°C)	18.2	16.3	14.7
Dissolved Oxygen (%)	101.5	100.7	91.8
Dissolved Oxygen (mg/L)	9.51	9.81	9.26
Conductivity (µs/cm)	898	845	881
pH	8.30	8.22	8.05

Table 3. Alkalinity sample results analyzed by Lower Colorado Regional Laboratory

Parameters	Results
Carbonate (CO ₃ ²⁻)	3.84 ppm
Bicarbonate (HCO ₃ ⁻)	166 ppm
Alkalinity	139 ppm
pH	8.3

2.2.2 Apparatus Field Testing

All veligers in all replicates survived exposure to each of the five pulsed voltage tests and the one constant voltage test. Veligers were alive immediately after exposure and at the 1-hour and 24-hour observation times. All veligers in the controls also were also alive at each observation. There were no notable behavioral changes observed.

Tables 4 and 5 are summaries of the electrical apparatus parameters tested in the field on live quagga veligers using pulsed voltage and constant voltage, respectively. Three additional parameters are recorded for tests involving pulsed voltage: the frequency and width of the pulse, and the

function generator frequency used to create the pulse. The peak-to-peak voltage is also recorded, as described in the methods section of this report. Exposure time is the length of time voltage was applied and was varied from one minute up to one hour.

In all tests, the distance between plates was kept a constant leaving enough gap in between to accommodate the height of a glass petri dish containing the live quagga veligers.

Table 4. Electrical apparatus output for the pulsed voltage tests

Test Number	Replicate Number	Voltage (Vpp. Oscope)	Exposure Time (minutes)	Distance between plates (cm)	Pulse frequency (kHz)	Pulse width (%)	Function Gen. Frequency
1	1	3.3	1:00	1.5	22.5	58	1-kHz
1	2	3.28	1:00	1.5	18	60	1-kHz
1	3	2.14	1:00	1.5	21.3	58.5	1-kHz
2	1	1.7	1:30	1.5	22	59	3-kHz
2	2	1.4	1:13	1.5	24.6	58.6	3-kHz
2	3	1.2	1:30	1.5	24.7	60.2	3-kHz
4	1	3	1:00	1.5	9.6	53.8	100-Hz
4	2	2.9	1:09	1.5	9.5	54.5	100-Hz
4	3	2.58	1:00	1.5	9.7	51.3	100-Hz
5	1	2.99	10:00	1.5	9.4	54.5	100-Hz
5	2	3.01	10:00	1.5	9.9	58.8	100-Hz
5	3	3.01	10:00	1.5	9.65	56.7	100-Hz
6	1	3.05	1:00:00	1.5	9.6	58.2	100-Hz

Table 5. Electrical apparatus output for the constant voltage tests

Test Number	Replicate Number	Voltage (Vpp. Oscope)	Exposure Time (minutes)	Distance between plates (cm)
3	1	1.1	1:00	1.5
3	2	1.1	1:00	1.5
3	3	0.95	1:00	1.5

3. Discussion

A key objective of the project was completed with the design and construction of the electrical apparatus. However, field testing was limited due to travel restrictions imposed on research projects for more than two years. A single field visit was made during the spring of 2022 to perform testing of pulsed and constant voltage fields using live quagga mussel veligers collected from nearby Lake Mead, NV. It would be desirable to perform more field testing before a full assessment of this technology can be made. For this reason, a new proposal was submitted to S&T to continue another year of research for this project. Funding status is not expected until October 2022.

3.1 Initial Field Testing

As described, field testing was performed on live quagga mussel veligers in Boulder City, NV during the spring of 2022. Results were not promising across the range and values of electrical field parameters incorporated in the bench-top electrical apparatus. The parameters set up and the range of values for these parameters were originally modeled after those found in the literature for food pasteurization due to its wide acceptance and commercial availability. However, there are some key differences that may explain the different results received during the field testing:

1. Quagga mussel veligers are larger multi-cellular organisms compared to bacteria that are the targets during food pasteurization. It's possible that the range of voltage set in the electrical apparatus may not be large enough to take into account the larger number of cells present in veligers.
2. Quagga mussel veligers have developing shells composed primarily of calcium carbonate. It is uncertain if shells play any role in inhibiting the effect of an electrical fields and no literature was found describing shells as possible barriers to electrical fields. This may require further investigation.
3. While pulsed electrical fields was selected as the electrical method to explore in this project for its adaptability to Reclamation facilities (e.g., small diameter pipelines), it may not be the most effective method of inhibiting veliger settlements inside of Reclamation facilities. Other literature has indicated the electrical current applied to the water may be more effective and should be further investigated.

3.2 Problems and Resolutions

3.2.1 COVID-19 Travel Restriction

Live veligers are not found in Colorado where the TSC is located. As invasive species regulated under the Lacey Act, the intentional transport of zebra and quagga mussels across state lines is illegal. Therefore, it is necessary to travel to and test at a location where live veligers are found. The research team selected Boulder City, NV where the laboratories for the Lower Colorado Regional Laboratory and Multi-species Conservation Program are located. Quagga mussels have been present in nearby Lake Mead since 2007 and can easily be collected for testing back in the laboratories in Boulder City.

However, for more than two years beginning in March 2020 when the COVID-19 pandemic became widespread in the United States, travel by TSC personnel was limited to mission-critical activities. Research projects funded by the S&T office were deemed to not to be mission-critical and travel was restricted. It was not until travel restrictions became relaxed in 2022 that we were able to travel for the first time to perform field testing with our electrical apparatus. Unfortunately, 2022 was also out closeout year for this project.

If this project continues, one possible solution is to use other zooplankton species as a surrogate for zebra and quagga veligers. *Daphnia* may be a good candidate. *Daphnia* are small planktonic crustaceans sometime called water fleas. The two most common species of *Daphnia* are *D. pulex* and *D. magna*. They are slightly larger in size than zebra and quagga mussels. They also do not have

developing shells. However, live *Daphnia* are not regulated as invasive species, can easily be ordered at pet supply stores, and laboratory procedures for keeping them alive until testing are straightforward.

3.2.2 Inadequate Timeline

As described above, travel for research projects was restricted and it was more than two years before field testing could first be performed on live quagga mussels. As a result, the timeline was largely compressed to the last year of this project. In normal years, field testing would have involved much larger rounds of repeated testing over a wider range of parameters and conditions to determine the optimum set of conditions. Initial results would be assessed, and changes could have been made to the electrical apparatus. For example, it may be necessary to test at higher voltages since the initial set of voltages used did not appear to have any effect on live quagga veligers.

We have decided to closeout this project in 2022 to serve as a good stopping point. Given the results of the initial (and only) field testing performed in Boulder City, we have submitted a new proposal to consider other electrical methods and approaches.

4. Conclusions

Over the course of this project to date, the following key objectives have been completed:

1. Literature review to identify potential applications of electrical methods to prevent or mitigate zebra and/or quagga mussel veliger settlement within small diameter pipelines in Reclamation facilities.
2. Selection of a single electrical method (pulsed electrical fields) that would be most adaptable to small-diameter pipelines in Reclamation facilities.
3. Design and construction of a bench-scale electrical apparatus where a petri dish containing live zebra or quagga mussel veligers may be subjected to pulsed and constant voltage fields.
4. An initial field testing using this bench-scale electrical apparatus on live quagga mussel veligers collected from Lake Mead, NV.
5. Development and submission of a new S&T proposal outlining accomplishments and results to date to continue one more year of research on this project.

The electrical apparatus, as constructed and without the benefit of further refinements that could have been afforded by further rounds of testing, did not provide meaningful results during the initial and only live quagga mussel testing performed in Boulder City, NV during the spring of 2022.

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Appendix A

Standard Operating Procedures

Disclaimer

This documentation may not answer every single question that could arise. If any questions remain, please do not hesitate to contact Tyler Stuhldreier at (720) 498-3358 or Jacob Lapenna at (303) 445-2829 for assistance.

Overview

There are two electrical boxes that are part of the entire apparatus: the high voltage (HV) generator and the test chamber. The HV generator contains all the electronics necessary to generate HV continuously or in pulses. Operation in both pulsed and direct current (DC) mode will be discussed in their respective sections. The test chamber contains metal plates that the HV is applied across, and samples of Petri dishes can be placed between them. The test chamber comes with a built-in safety feature that allows for power to be immediately cut if the test chamber is opened during operation, preventing direct exposure to HV within the test chamber if the HV generator is not first turned off.

Equipment

The apparatus in its entirety consists of the test chamber, the HV generator, a function generator, a DC power supply, and an oscilloscope.

HV Generator

The HV generator as it will be used is depicted in Figure 1. There is only one power cord associated with it. Note that Figure 1 shows the proper orientation for the HV generator when it is laying down or standing up. The HV generator handles the generation of the HV signal that is applied to the test chamber.

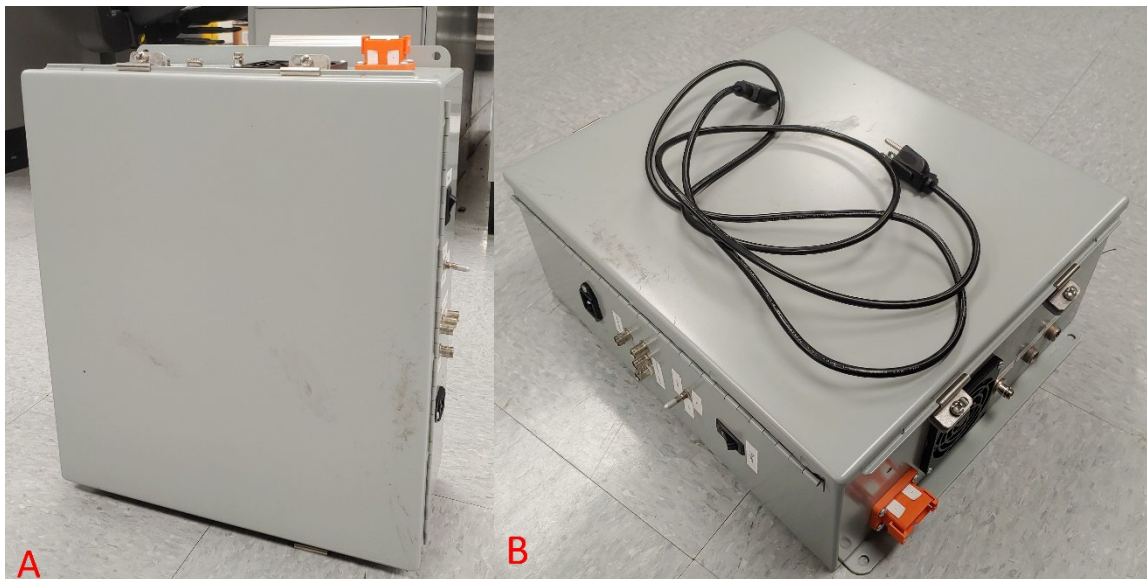


Figure 1 – The HV generator in a standing up position (A) and in a laying down position with the power cord (B).

Test Chamber

The test chamber looks like the HV generator except it has multiple cables attached directly to it and has large grey tabs that can be rotated to lock/unlock the chamber as shown in Figure 2. The

test chamber is where samples of petri dishes can be subjected to high voltage to study the effects of high voltage pulses on populations.

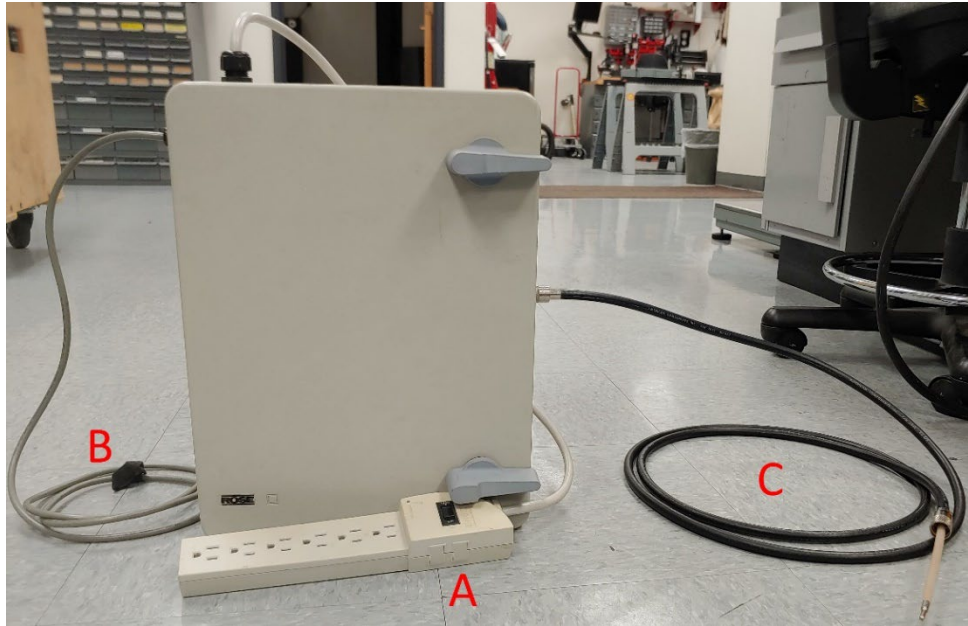


Figure 2 – The test chamber from the front side. Note the power strip (A), power cord (B), and high voltage cable (C).

Function Generator

The function generator is used to generate the necessary signal for the HV generator's oscillator circuit to work and is shown in Figure 3. The frequency of the signal generated by the function generator will primarily determine the frequency of pulses in the test chamber. A coaxial cable is required between the function generator output and the PULSE_INPUT terminal of the HV generator.

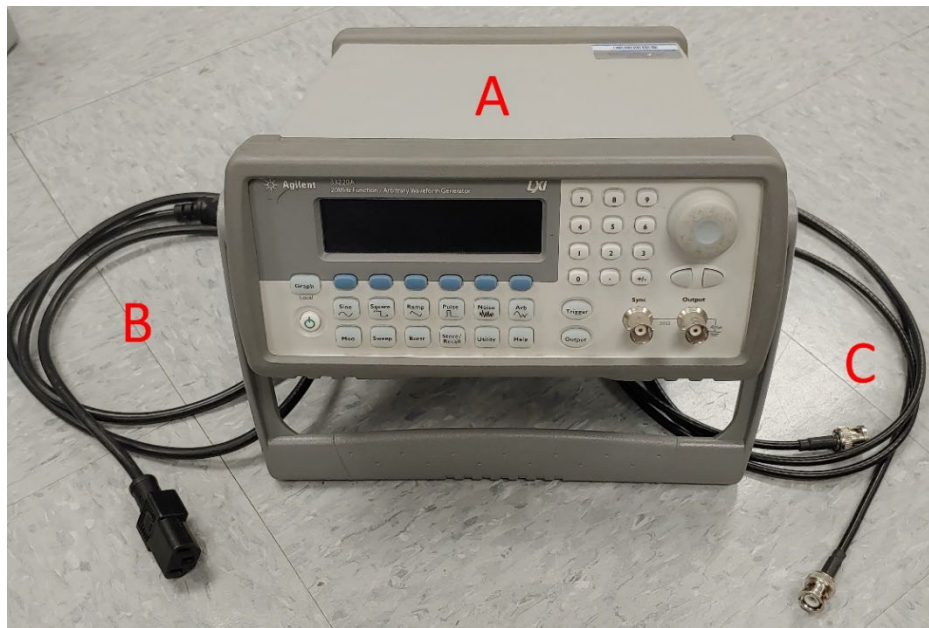


Figure 3 - Function generator (A), its power cable (B), and a coaxial cable (C).

DC Power Source

The DC power supply is connected to the HV generator and is the primary power source that is switched to generate high voltage. Adjusting the voltage output of this source will increase or decrease the magnitude of the voltage generated by the HV generator. It consists of the source itself with two cables connected to it as shown in Figure 4.

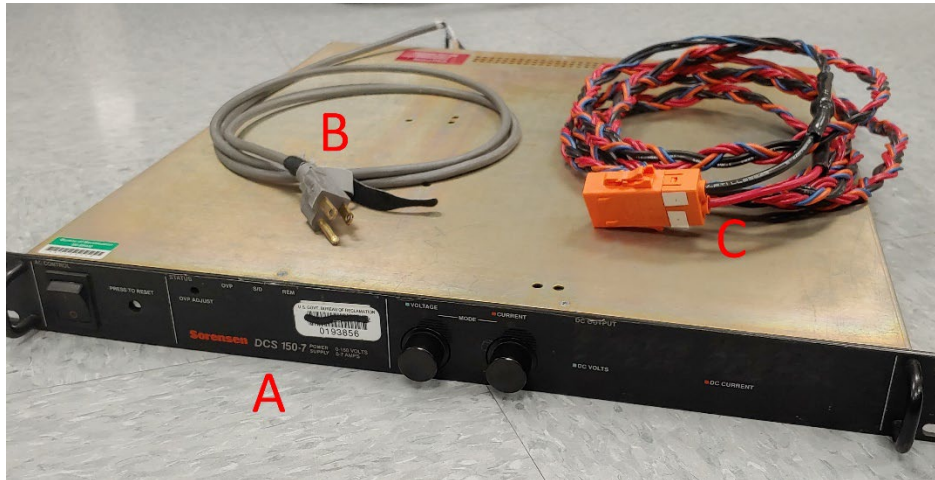


Figure 4 - DC power source (A), its power cable (B), and the connection to the HV generator (C).

Oscilloscope

The oscilloscope is used to monitor various signals from the HV generator including the output and is shown in Figure 5. The oscilloscope is accompanied by four coaxial cables so that all four channels of the oscilloscope may be used at once as well as an AC test adapter on its power cord. Usage of the oscilloscope alongside the apparatus will be discussed in detail so that a user can understand if the apparatus is working and the magnitude of the output voltage.

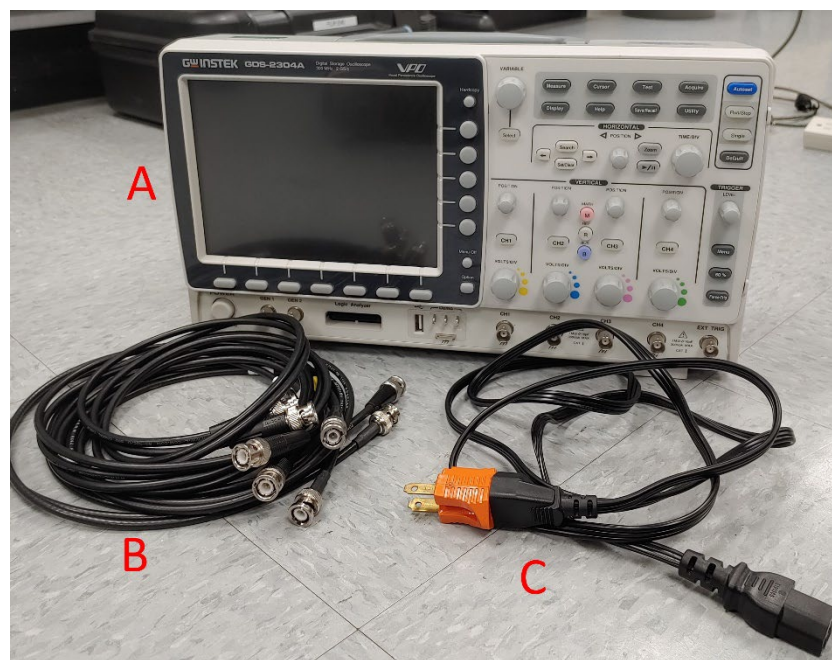


Figure 5 – Oscilloscope (A), coaxial cables (B), and its power cord terminated with an AC test adapter (C).

Apparatus Setup

The entire apparatus sources its power from a single 120 V wall outlet connection through the test chamber. This means the goal is to get all necessary components plugged into the test chamber surge protector.

To setup:

- 1) Ensure all pieces of equipment are not connected and are in the off position. The HV generator should be shut with the metal tabs and screws along the perimeter of the electrical box (Figure 6) and the Power switch in the off position. The function generator and DC power supply should have their power in the off position and be unplugged. The test chamber should be left open and should be unplugged from any outlets.

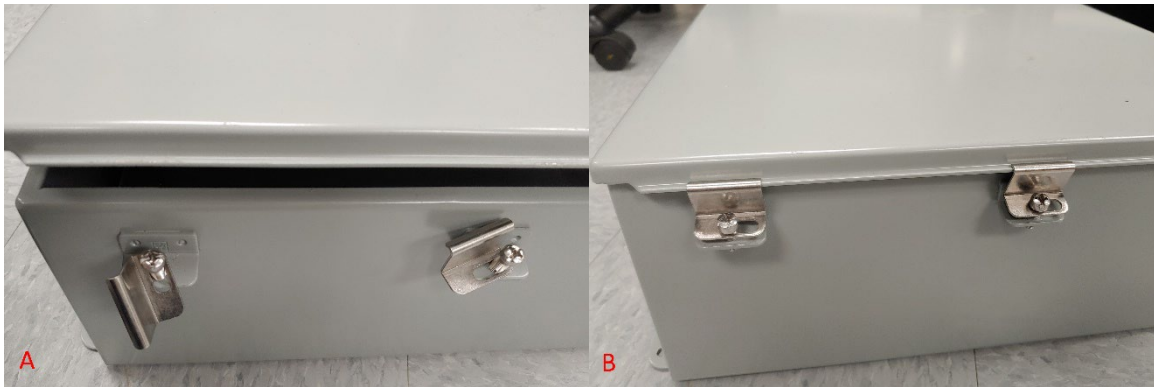


Figure 6 – HV generator shut with metal tabs open (A) and closed (B).

- 2) Using their power cables, plug the DC power supply, function generator, and HV generator into the AC surge protector that is a part of the test chamber (Figure 7). The surge protector's power is connected through a relay that is engaged when the test chamber is closed, preventing power flow to the DC power supply, function generator, and HV generator when the test chamber is opened. The surge protector should remain in the OFF position. The oscilloscope can optionally be plugged into this surge protector as well or it can be powered from its own outlet.

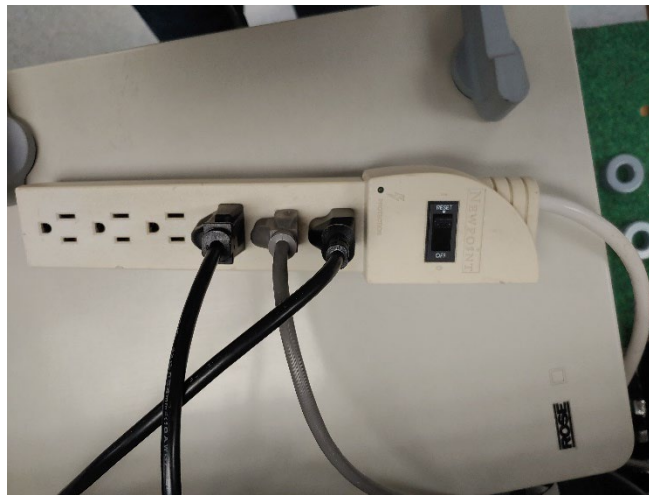


Figure 7 - AC surge protector on the test chamber with the HV generator, DC power supply, and function generator plugged in.

- 3) Take the end of the HV lead that is part of the test chamber and insert it into the HV OUTPUT terminal of the HV generator (Figure 8). Insert until the lead can be inserted no longer, and then twist the cable onto the HV OUTPUT terminal.



Figure 8 – End of the HV lead on the test chamber (A), inserting it into the HV OUTPUT (B), and inserted (C).

- 4) Take a coaxial cable that is with the function generator and connect one end to the Output on the function generator (Figure 9) and the other end to PULSE INPUT on the HV generator (Figure 10).

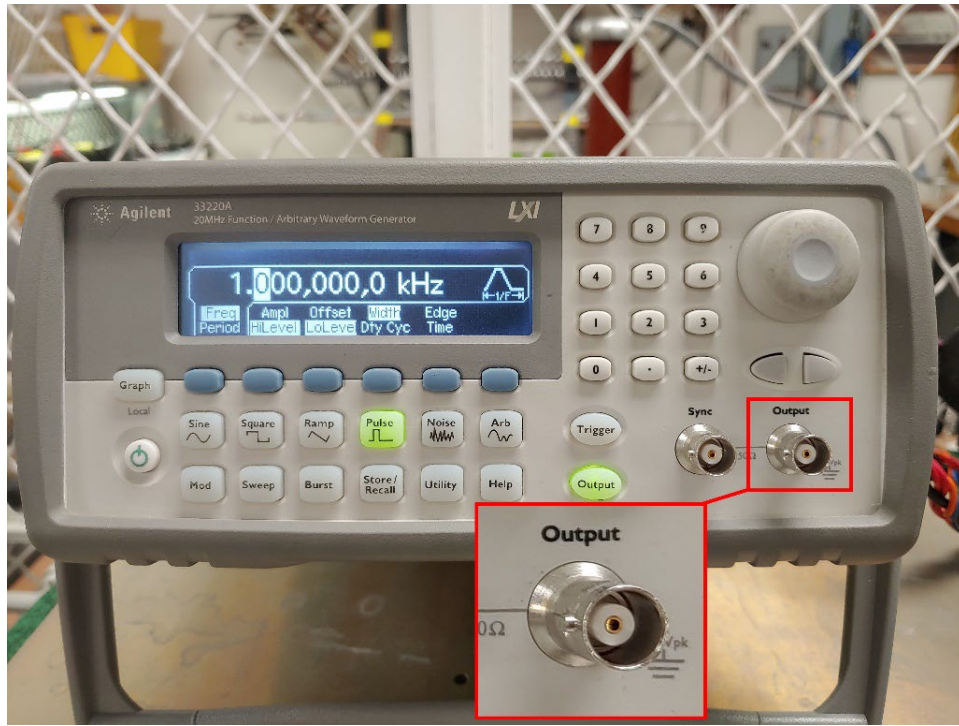


Figure 9 – Function generator output.

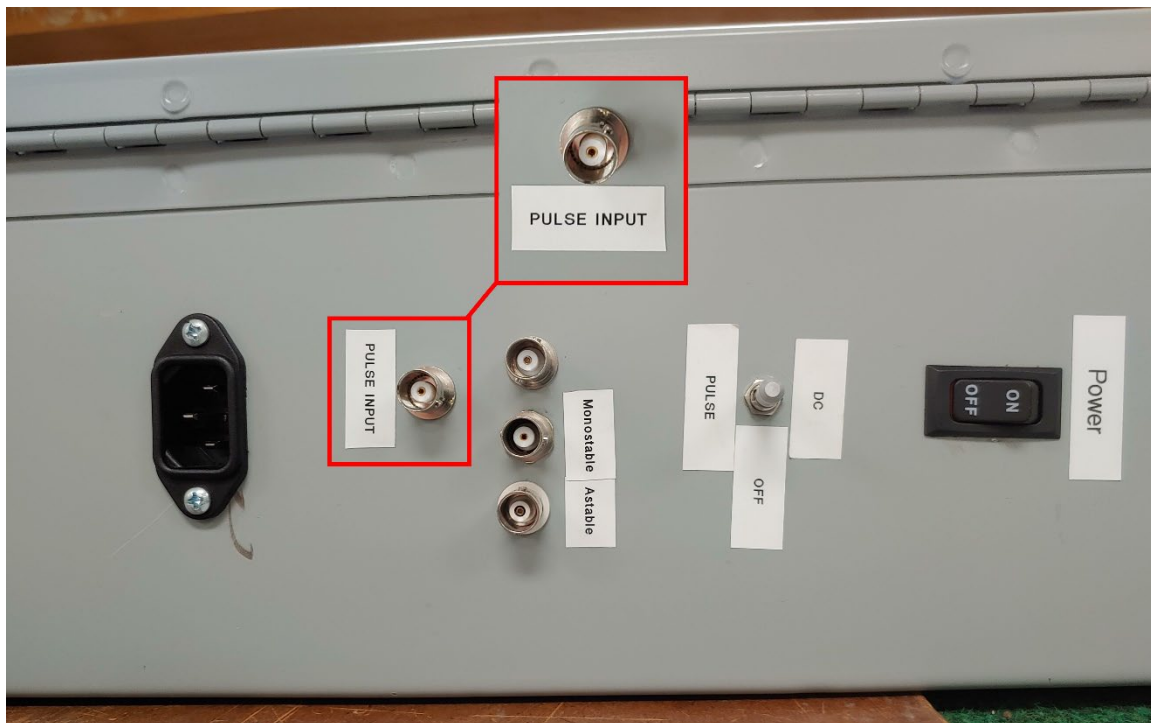


Figure 10 – PULSE INPUT on the HV generator.

- 5) Take a coaxial cable from the oscilloscope and connect one end to the desired channel (CH3 recommended) on the oscilloscope (Figure 11) and connect the other end to OUTPUT on the HV generator (Figure 12). It is additionally useful to connect another coaxial cable between the Astable port on the HV generator (Figure 13) and a channel of the oscilloscope (CH2 recommended). For details regarding the coaxial cables and connecting them to the oscilloscope, see **Oscilloscope Setup**.

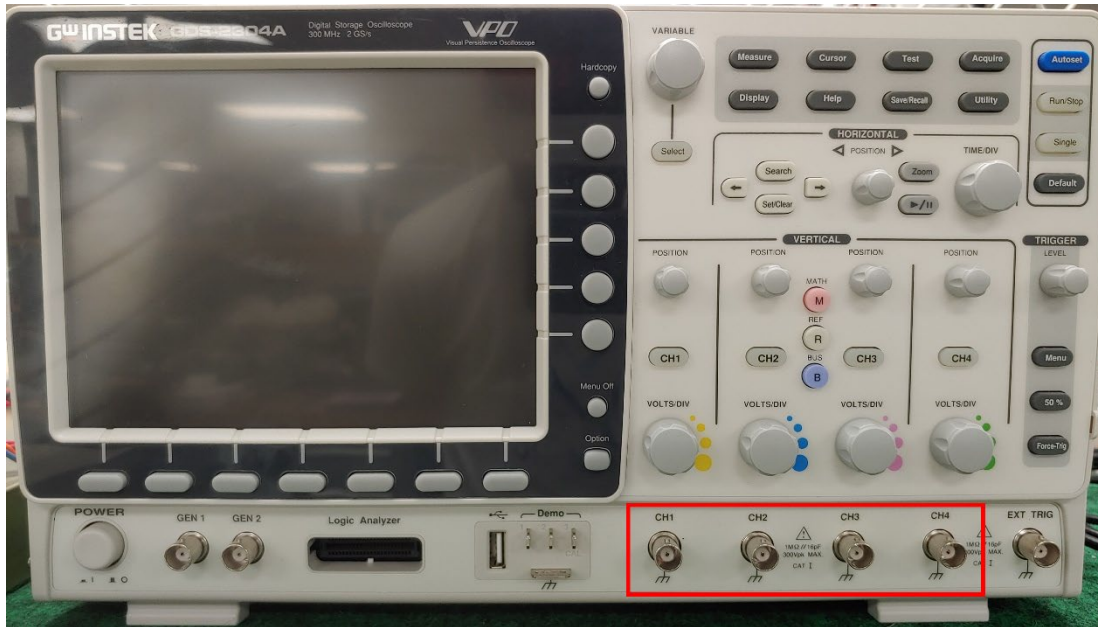


Figure 11 – Input ports on the oscilloscope.

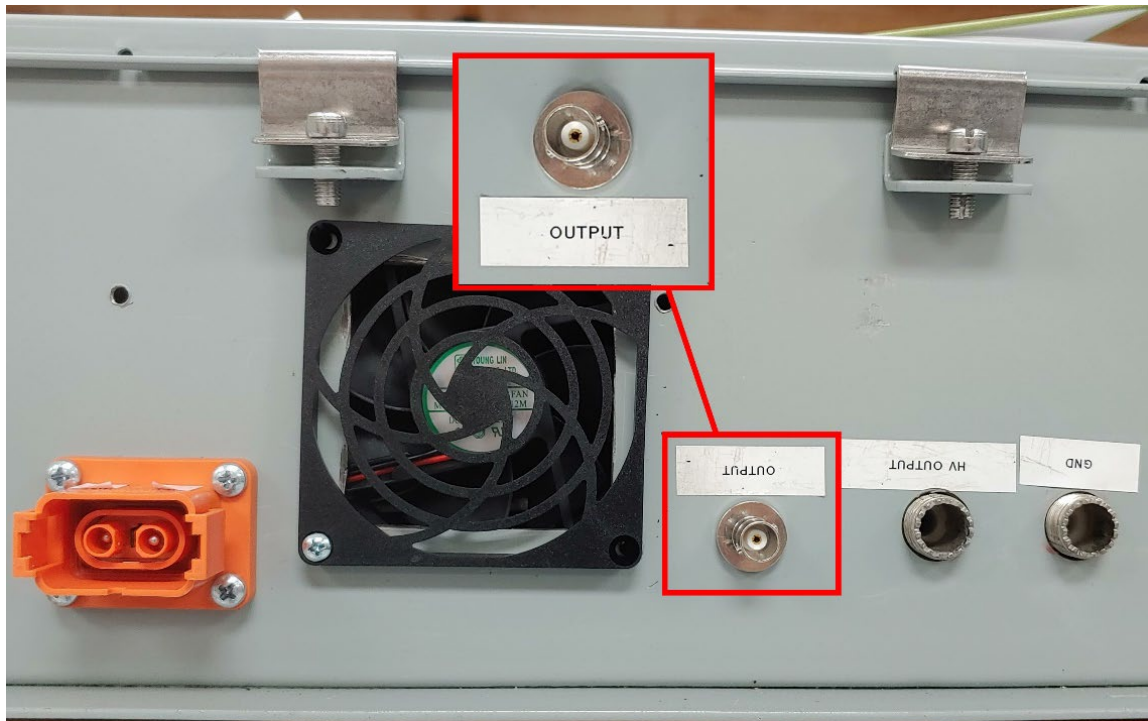


Figure 12 – Output port on the HV generator.

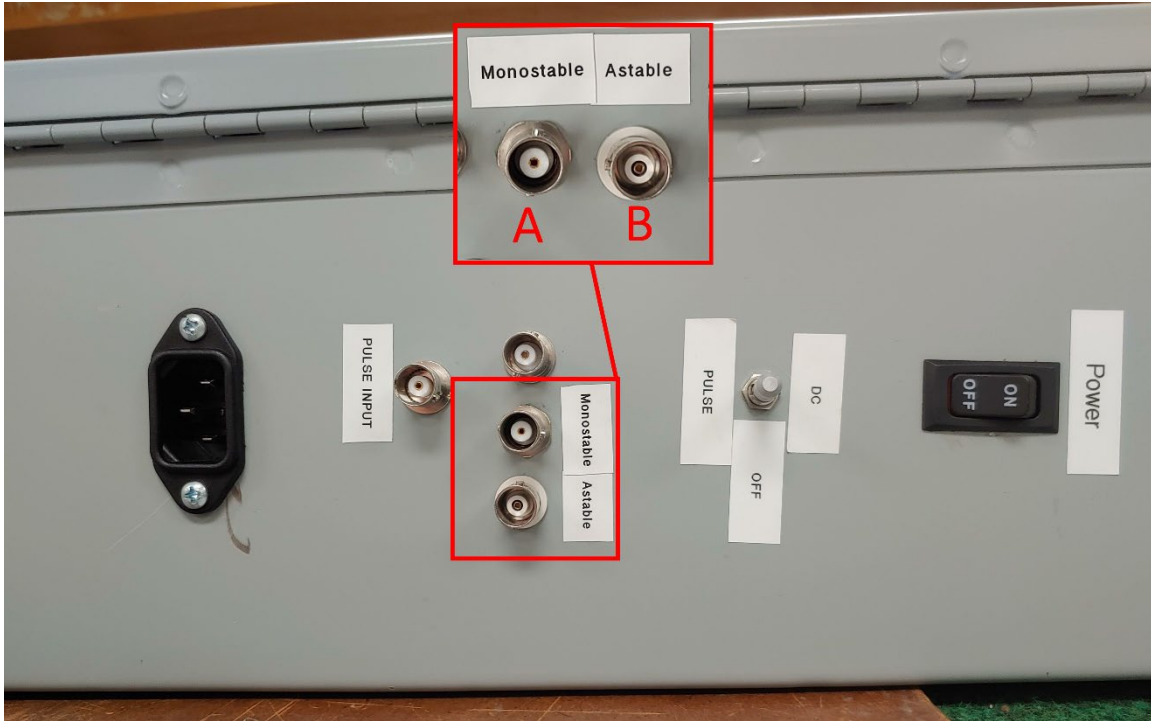


Figure 13 – Monostable (A) and Astable (B) test ports used for seeing the respective signals on the oscilloscope.

- 6) Plug the DC power supply into the HV generator using the colored cable ending in an orange termination. Ensure that the polarity marks line up between the cable and the HV generator (Figure 14) otherwise major issues will arise.

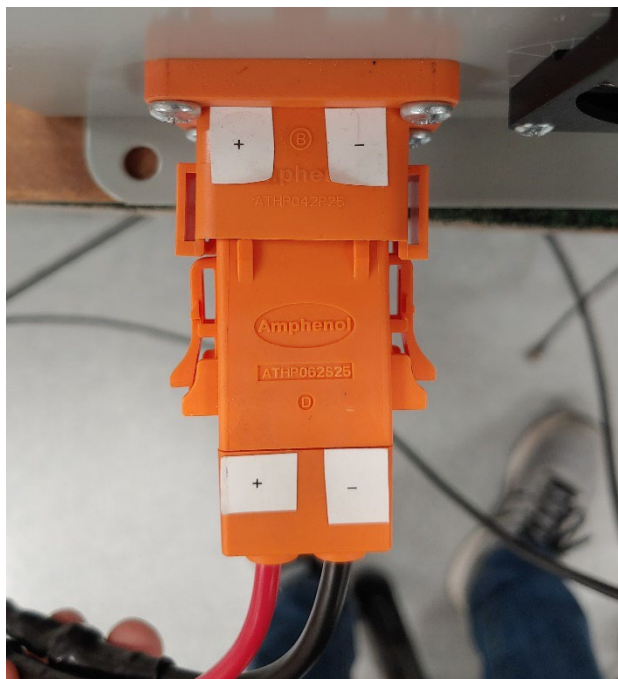


Figure 14 – Connection between DC source and the HV generator. Polarity must match.

- 7) Plug the AC plug on the test chamber into a three-prong 120 Volt wall outlet. Switching the surge protector to RESET and turning on the function generator, DC power supply, or HV generator will now provide power to each individual device. It is recommended to turn on each device only when necessary.
- 8) Make necessary changes to the test chamber specimens now before proceeding with the HV generator. In the apparatus' current setup state, nothing is connected to a power source. Ensure that samples are placed as central to the plate area as possible as there are edge effects of the electric field near the edges of the plate area. These edge effects make the electric field much less predictable in these regions when compared with the center. **Take care to ensure the metal plates will not contact one another during operation or the high-voltage circuit will be shorted and damage to the apparatus may result.**
- 9) Close the test chamber by closing the lid and turning the gray latches on the front panel clockwise as shown in Figure 2.

The apparatus is now setup and ready to be turned on. As a double check, the only thing that should be plugged into an AC outlet is the test chamber's AC plug and all parts of the apparatus should be closed shut.

Turning the Apparatus On

Before generating the high voltage pulses, everything should be setup to first generate a low voltage output. This requires setting up the function generator and ensuring that the oscillator circuit is working properly and checking for an output on the oscilloscope. For more details on working with the oscillator circuit, see the separate documentation file *Oscillator Documentation*.

To get a low voltage output:

- 1) Turn the function generator ON. The preferred output from the function generator is a pulse with the following settings (Figure 15):
 - Frequency - 1 kHz
 - Hi Level - 3 V
 - Lo Level - 0 V
 - Width - 40 μ s

To change a setting, press one of the blue soft keys under the function generator display. You want to press the one that is under the setting you are trying to set. For example, to set the Frequency press the blue key under Freq/Period until Freq is selected. Then, use the number pad on the function generator to set the magnitude and the blue soft keys under the display for the preferred units.

Since the frequency from the function generator dictates the frequency that the HV pulses will occur, this can be changed as needed. Once the function generator output is setup, you may output the signal from the function generator (Figure 16).

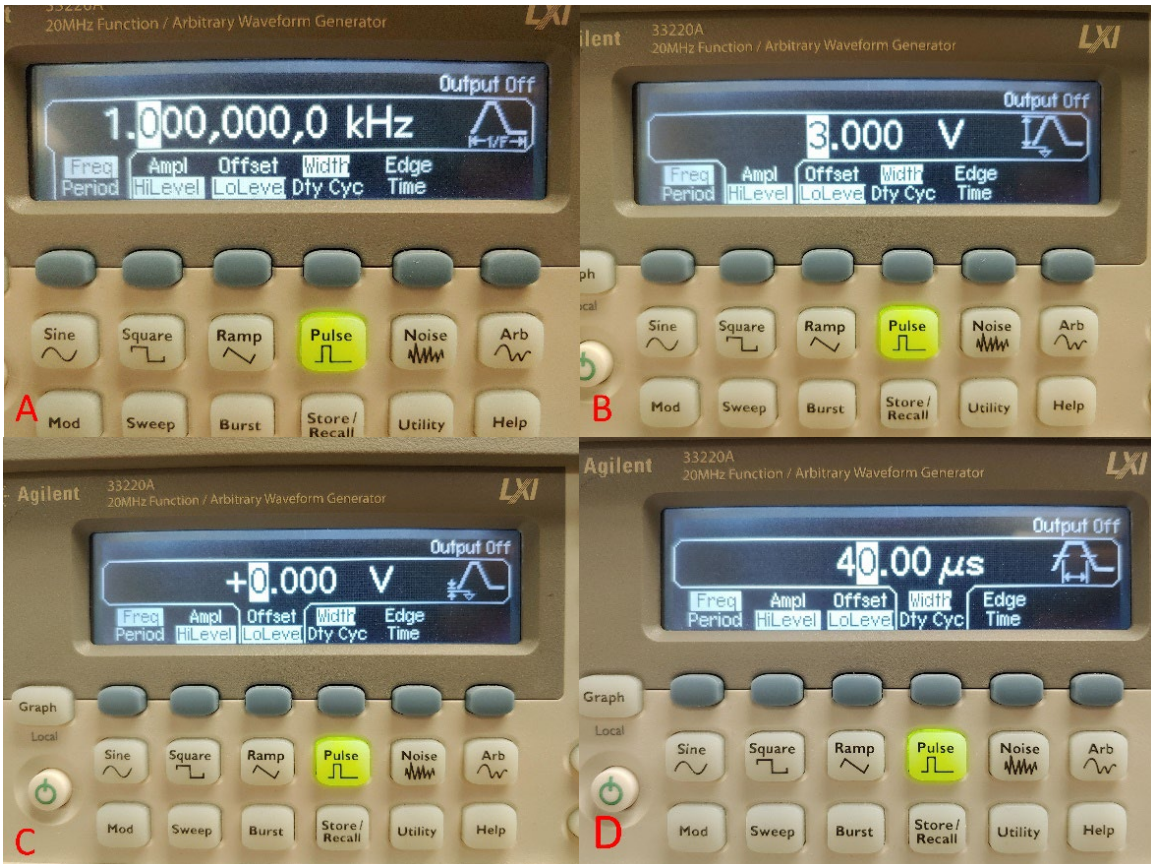


Figure 15 – Frequency (A), Hi Level (B), Lo Level (C), and Duty % (D) settings of the function generator.

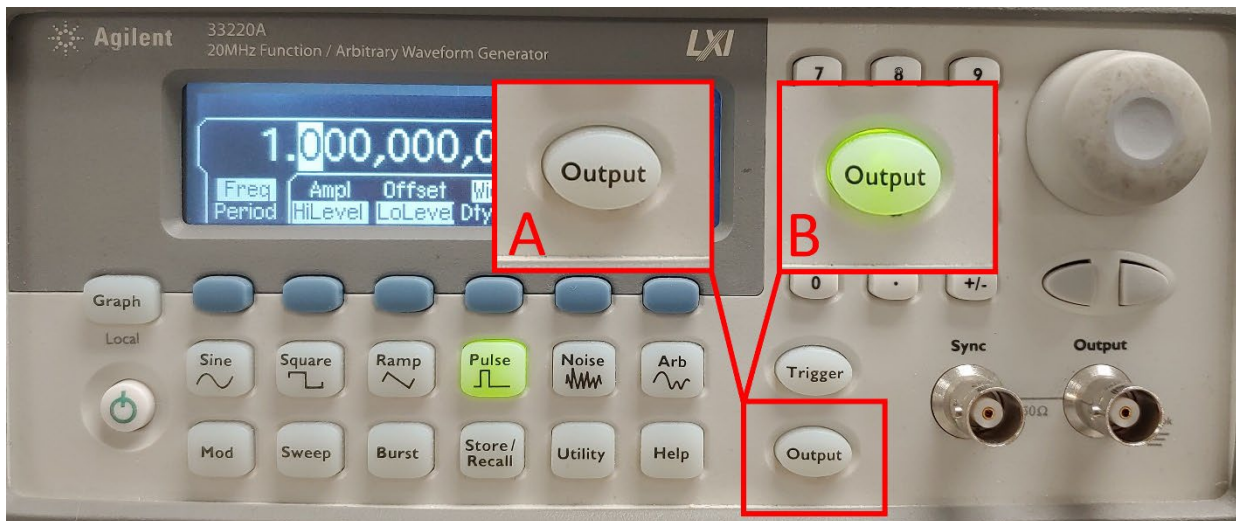


Figure 16 – Output button of the function generator off (A) and on (B).

- 2) Make sure the toggle switch on the HV generator (Figure 17) is set to the middle OFF position and not in PULSE or DC mode. Turn ON the HV generator with the rocker switch.

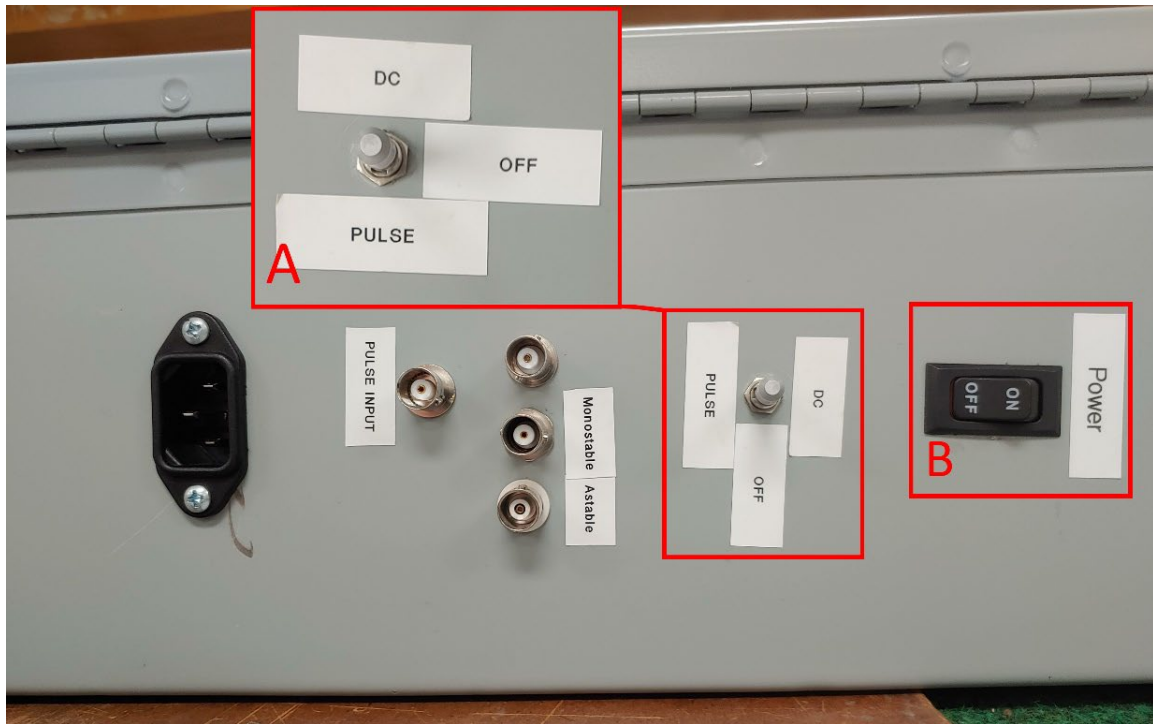


Figure 17 – HV generator toggle switch in the OFF position (A) and power switch (B)

- 3) It is assumed that the oscillator circuit has been tuned while following this procedure, but if there is any doubt in how it has been tuned, please see *Oscillator Documentation* for how to tune the oscillator circuit before proceeding.
- The oscillator is considered tuned when the astable signal has a frequency in the range of 20 – 25 kHz and the monostable signal has a pulse length approximately equivalent to one period of the astable signal. The frequency and pulse length of these signals can be measured on an oscilloscope connected to the ASTABLE and MONO ports on the HV generator. See **Oscilloscope Setup** to understand how to view a signal and measure its properties.
- 4) Before turning the DC power supply on, turn the VOLTAGE knob (Figure 18) counterclockwise all the way to its manual stop. This ensures that the voltage can be manually increased from 0 V when the power supply is turned on and does not inadvertently apply a larger than intended voltage to the specimen.



Figure 18 – Voltage knob (A) and power switch in the off position (B) on the DC power supply.

- 5) Turn the DC power supply ON by switching the AC Control rocker switch from O to |. (Figure 18).

The apparatus is now on and ready to be used for experimentation. No high voltage output will be available until the HV generator is switched to PULSE or DC modes and the DC power supply is supplying voltage. To supply voltage, turn the VOLTAGE knob (Figure 18) clockwise. It is not recommended to turn the DC voltage up until one is finally ready to get the output. Switching the HV generator into PULSE and DC modes is done via the toggle switch with labels OFF, DC, and PULSE (Figure 17). To change the position of this toggle switch, you must first pull the toggle switch up and then move it to the desired position. Trying to move it directly to the position you want without pulling it up first will not work and could damage the switch.

It is recommended that the oscilloscope is setup to measure the output before initiating any output. The following sections **Oscilloscope Setup** and **Measuring the Output** will explain setup and use of the oscilloscope with regard to the output signal.

Oscilloscope Setup

Determining useful quantities from the HV generator requires the use of the oscilloscope. An oscilloscope is simply a device for visualizing voltage signals through time.

Only a single channel of the oscilloscope must be used to determine the voltage output in the test chamber, but other signals from the HV generator can be displayed alongside the output and it is useful to monitor them even though they are not exactly the quantities of interest. The BNC ports under the toggle switch on the HV generator labeled MONOSTABLE and ASTABLE (Figure 17) can be used to show the monostable and astable signals from the oscillator circuit.

When the oscilloscope is turned on it will usually start up with whatever settings were last used. This means that this entire section can be skipped if one is confident that the oscilloscope is already setup using these instructions. They include information about how to correctly display a signal using the various axis scales and trigger settings as well as how to complete measurements using two different techniques: Measure and Cursor.

No signal on a channel is displayed until the respective channel button is pressed and its LED is lit up as shown in Figure 19. Channel 1 is displayed in yellow, Channel 2 in cyan, Channel 3 in magenta, and Channel 4 in green.

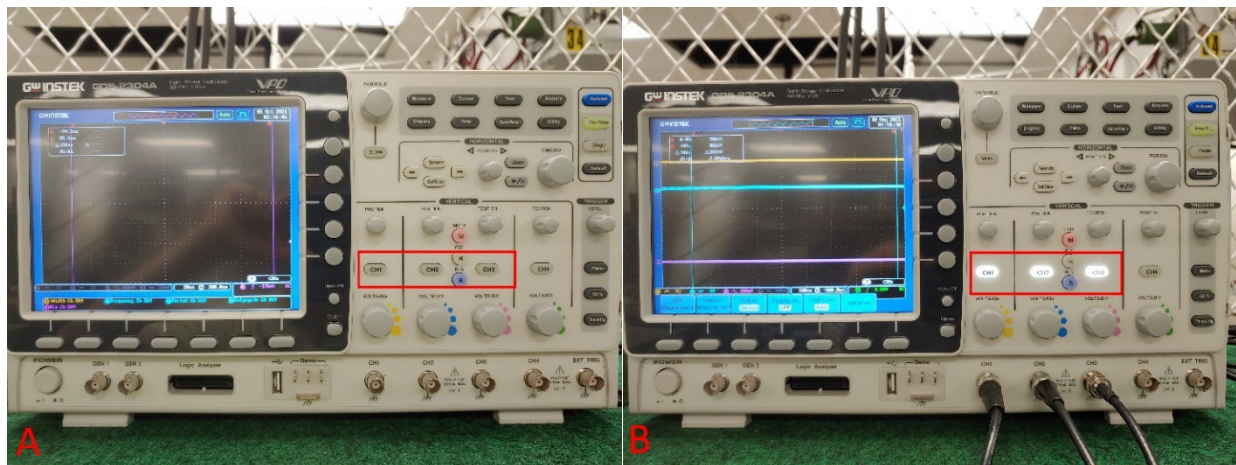


Figure 19 – Oscilloscope channels off (A) and on (B).

Figure 20 and Figure 21 will outline the major functional buttons on the oscilloscope necessary for use as well as the information on the digital display, respectively. An attempt has been made to reference back to these images within the instructions, but it is instead recommended that one familiarize themselves with where the different functional buttons are and the different aspects of the display.

Figure 20 shows the major functions that will be used on the oscilloscope. They are:

- A) Variable Knob and Select – Allows for selecting different menu options.
- B) **Measure** – display measurements of the signals.
- C) **Cursor** – cursors on the screen can be used to measure the peak of the output.
- D) Run/Stop – Resumes/Pauses the acquisition of new images.
- E) Time/Div Knob – Sets the amount of time per division on the oscilloscope screen. Total time displayed is ten times the Time/Div setting.
- F) Vertical Position Knob – Can change the vertical position of the channel's signal.
- G) Channel Display On/Off Buttons – Turns the display of that channel on/off. See Figure 19.
- H) Volts/Div Knob – Sets the number of volts per division on the oscilloscope screen.
- I) Trigger Level Knob – Sets the trigger level for which the oscilloscope will display a clean signal that is being triggered off.
- J) **Trigger: Menu** – Change trigger settings including which channel is being triggered off.

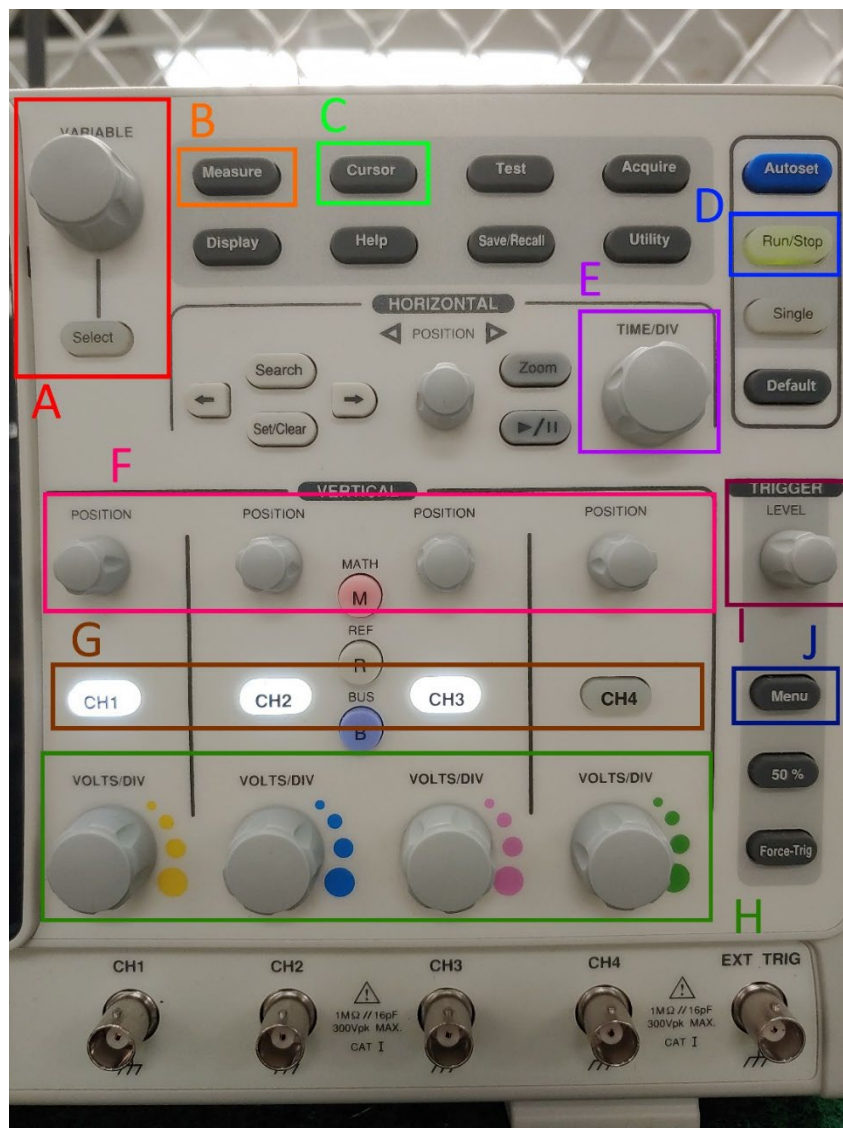


Figure 20 – Major functional buttons and knobs on the oscilloscope.

Figure 21 shows the important areas of the oscilloscope screen. They are:

- A) Main display area – Where signals are displayed
- B) Cursor information – Reports the location of the cursors and the difference between them.
- C) Cursors – Can be moved to determine the magnitude of volts (vertical) and time (horizontal) and more.
- D) Signals – The signals that we want to see.
- E) One division of the screen – fixed areas of the display whose size is dependent on the Volts/Div and Time/Div Settings.
- F) Measurements – Displayed when a measurement is active.
- G) Volts/Div Setting – Sets the vertical scale of the divisions of the screen.
- H) Time/Div Setting – Sets the horizontal scale of the divisions of the screen.
- I) Trigger Level Settings – Describes the channel for which the oscilloscope is triggered off of and the magnitude of voltage required to trigger.
- J) Horizontal Soft Keys – Allows access to different context-dependent menu options when a hard key (**Measure, Cursor, Trigger: Menu**) is pressed.
- K) Vertical Soft Keys – Allows access to different context-dependent menu options.

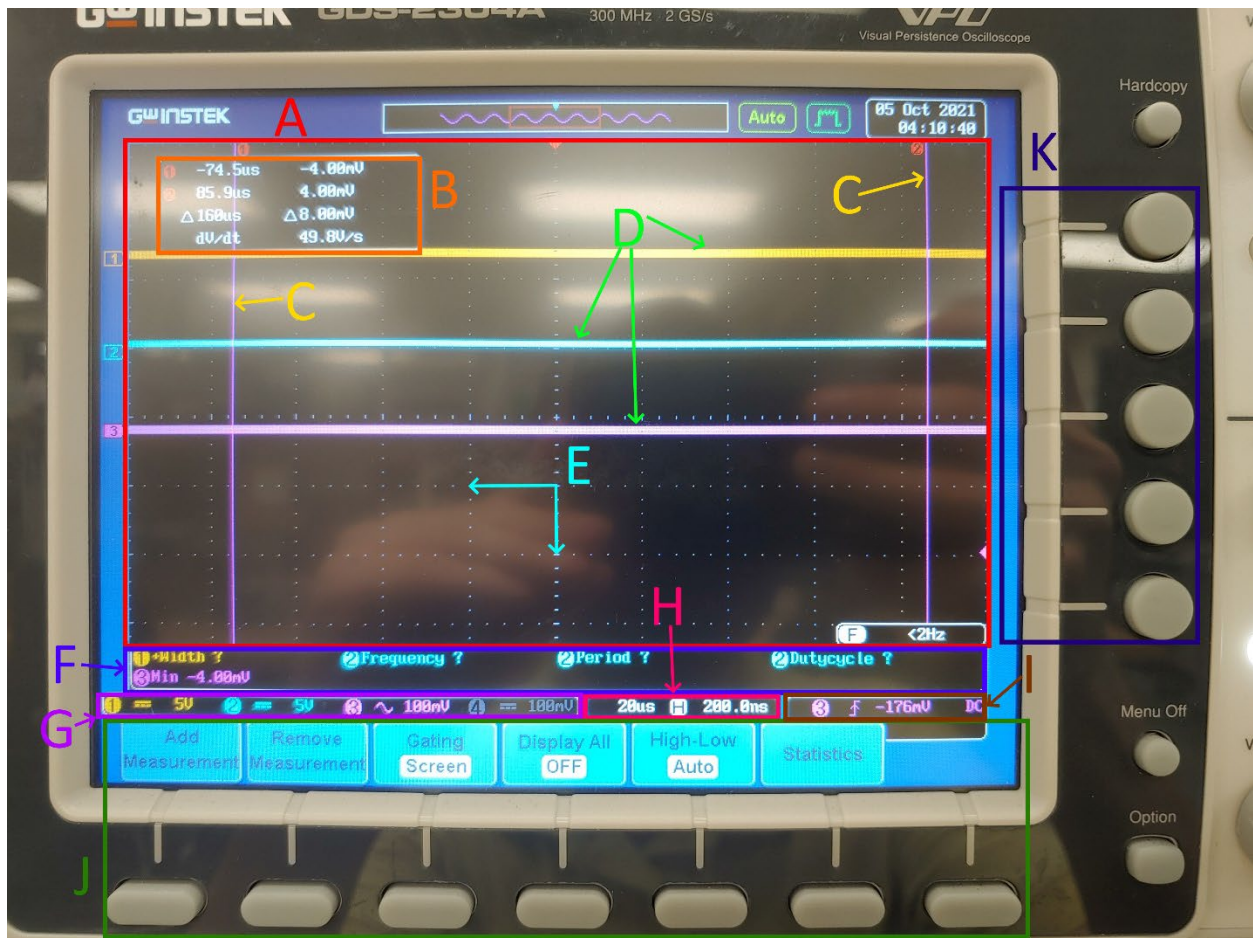


Figure 21 – Major areas of the oscilloscope screen.

The large area on the oscilloscope screen is divided by a series of lines forming a grid with 10 horizontal divisions and 8 vertical divisions. The horizontal axis corresponds with time while the vertical axis corresponds with voltage. Adjusting the Volts/Div knob (Figure 20, H) will change the number of volts per vertical division on the screen. The number of volts per division on the screen is the Volts/Div Setting and can be adjusted separately for each channel. For example, in Figure 22, Channel 1 (yellow) has a Volts/Div Setting of 1.0 V/Div and this means that each time the input signal on Channel 1 changes by 1 vertical division, it has changed by 1 volt. Likewise, the Time/Div knob (Figure 20, E) changes the amount of time per horizontal division on the screen. This setting is universal and cannot be adjusted per channel. The setting is displayed on the oscilloscope screen in Figure 21, H and is set at 50 μ s/Div and hence each time the input signal travels 1 horizontal division, 50 μ s has passed. The recommended Volts/Div and Time/Div settings are as follows:

- Volts/Div for non-output Channel: 5 V
- Starting Volts/Div for output Channel: 100 mV
- Time/Div Setting: 20 μ s

The Vertical Position Knobs for each channel can be used to reposition the signals vertically so that each signal can be viewed without another on top or underneath it.

Measuring the Output

The output voltage will depend on the mode that the HV generator runs in and the input voltage from the DC power supply. Running the HV generator in PULSE mode requires a larger input voltage from the DC power supply to achieve the equivalent peak-to-peak voltage that the DC mode generates. Thus, when running in DC mode, the input voltage from the DC source and the output should be monitored more carefully to ensure the high voltage generated is not too high.

The peak-to-peak voltage of the output can be measured directly on the oscilloscope using the cursors while the oscilloscope is running and not paused. The following instructions are critical for ensuring that the output signal is measured as accurately as possible.

Trigger

The Trigger Level settings, all accessed through the **Trigger:Menu** hard key (Figure 20, J) except for the Trigger Level which uses a knob (Figure 20, I), include:

- Type – type of waveform that will cause a trigger
- Channel – the channel which the oscilloscope is triggered from.
- Coupling – the mode of coupling
- Slope – Associated with the Edge Type, will look for either a rising, falling, or either edge.
- Level – the voltage quantity on the trigger channel required for a trigger. Can be separately adjusted using the Trigger Level knob.
- Mode – Always set to Auto
- Holdoff – minimum time required between triggers

These settings are summarized on the main display in Figure 21, I. To set the various settings, first press the **Trigger:Menu** hard key. This will bring up options for all of the above settings above the horizontal soft keys (Figure 21, J). Pressing most of these soft keys will bring up an additional

menu on the vertical soft keys (Figure 21, K), where pressing the vertical soft key will have one of three results which apply to setting any of the oscilloscope options, not just the Trigger settings. The results are:

- 1) The setting will simply change altogether. For example, pressing the Type horizontal soft key and then any of the vertical soft keys changes the Type setting altogether without need for any other input.
- 2) An additional menu may appear to the left of the setting you just selected. This requires use of the Variable knob (Figure 20, A) to change to the desired setting. One can then use either the vertical soft key or the Select button under the variable knob to select the desired setting. For example, pressing the Source horizontal soft key and then the uppermost vertical soft key will open a menu where you must select the desired channel using the Variable knob.
- 3) Nothing will happen. This is the case for vertical soft key menus with a clockwise arrow in them. These settings can be adjusted using the Variable knob without the need to select the respective vertical soft key. Simply opening this menu with the horizontal soft key will allow adjustment of the setting with the Variable knob. For example, pressing the Level horizontal soft key will bring up a vertical soft key menu where the trigger level (uppermost setting) can be adjusted using the Variable knob. Note that any time the Variable knob can be used to adjust something, the Select button under it lights up.

If a vertical soft key menu does not appear when pressing a horizontal soft key menu, then the settings are simply being cycled through. For example, when the Slope horizontal soft key is pressed, it simply select the next option shown. For these settings, just press the horizontal soft key menu until the desired setting is set.

These procedures apply to all hard key and soft key menus.

For measuring the output signal, the following settings are recommended:

- Type – Edge
- Channel – CH3
- Coupling – DC
- Slope – Rising
- Level – -100 mV
- Mode – Auto
- Holdoff – 10.0 ns

The trigger level should be adjusted as necessary to clearly see the output signal. It should be set to a negative quantity.

Cursor

Cursors will give information about the magnitude of the output signal and will be the easiest way to get a consistent measurement. The Measure hard key can also be used, but due to the instability of the signal the measurement will fluctuate a lot and therefore it is not recommended.

Pressing the Cursor hard key once will open the horizontal cursors menu. The soft key options will be:

- H Cursor – This soft key controls which cursors are actively being moved by the Variable knob. Pressing it while already selected will cycle through selecting both, only the left, and only the right cursors for movement. You can cycle through the options using the Select button under the Variable knob as well.
- H Unit – Soft key that controls the unit being used to measure the horizontal quantity. Will only use S here. Pressing this horizontal soft key will just cycle through the options.

Pressing the Cursor hard key a second time will open a horizontal soft key menu that includes new options for the vertical cursors as well. The descriptions of the horizontal settings still apply. The new options are:

- V Cursor – This horizontal soft key controls which cursors are actively being moved by the Variable knob. Pressing it when selected will cycle through selecting both, only the top, and only the bottom cursors for movement. You can cycle through the options using the Select button under the Variable knob as well.
- V Unit – Horizontal soft key that controls the unit being used to measure the vertical quantity. Will only use Base here. Pressing this horizontal soft key will just cycle through the options.

The properties of the cursors can be viewed in the main display area as shown in Figure 21, B. The main quantity of interest for measuring the output signal is ΔV , which is in the third row and second column on the display. The vertical cursors will be placed on the top and bottom peaks of the output waveform. Figure 22 is an example of the output being measured with the vertical cursors.

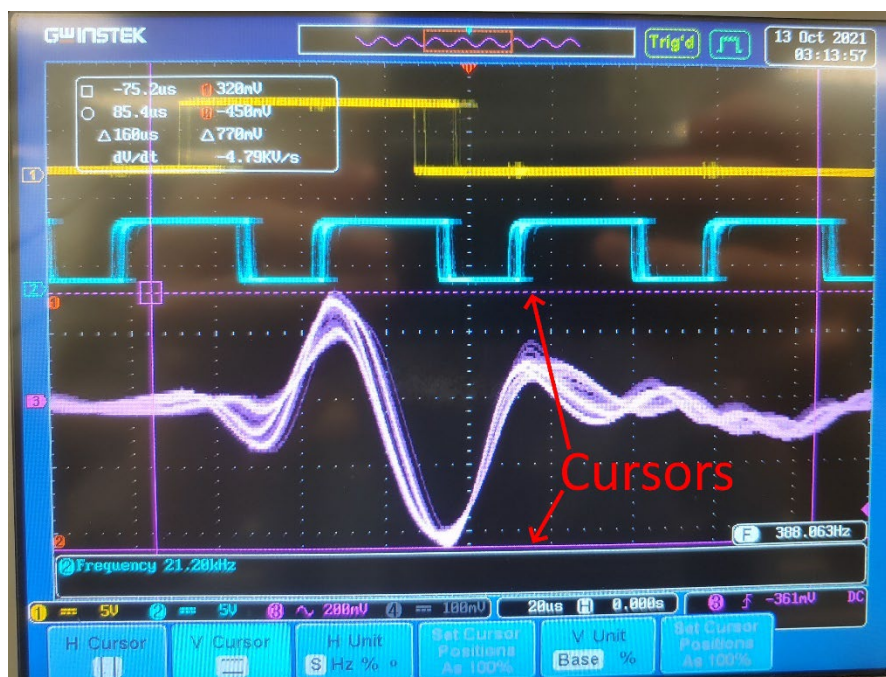


Figure 22 – Vertical cursors being used to measure the peak-to-peak voltage of the output signal.

Output

Table 1 gives a summary of the voltage generated in both PULSE and DC modes for given DC power supply inputs. This table should be used only as a reference and not for actual evaluation of the output voltage since it can vary with the oscillator pulse frequency and other unidentified factors. Note that the peak-to-peak voltage reported in the Output columns and seen on an oscilloscope are the result of a voltage divider, and the HV Output columns gives the actual voltage generated across the plates in the test chamber. Equation 1 gives the voltage divider relationship between the output and the actual high voltage generated in the test chamber.

Table 1 – Everything related to output voltage. Astable Frequency = 21.3 kHz, Duty % = 60%

DC Source Voltage (V)	Pulse Output (Vpp)	Pulse Output Between Plates (kVpp)	DC Output (Vpp)	DC Output Between Plates (kVpp)
1.0	0.0300	0.148	0.076	0.374
5.0	0.109	0.536	0.275	1.353
10.0	0.31	1.525	0.54	2.656
15.0	0.406	1.997	0.8	3.935
20.0	0.52	2.558	1.12	5.509
25.0	0.65	3.197	1.29	6.346
30.0	0.77	3.788	1.44	7.083
35.0	0.941	4.629	Arcing	Arcing
40.0	1.05	5.165	Arcing	Arcing
45.0	1.18	5.804	Arcing	Arcing
50.0	1.3	6.395	Arcing	Arcing
55.0	1.44	7.083	Arcing	Arcing
60.0	1.55	7.625	Arcing	Arcing
65.0	1.67	8.215	Arcing	Arcing
70.0	1.75	8.608	Arcing	Arcing

$$V_{chamber} = \frac{V_{output}}{0.000203292} = 12,767 \cdot V_{output} \quad (\text{Equation 1})$$

